THE R. KIMURA LABORATORY

AUTHOR(S):

Kimura, Ren

THE R. KIMURA LABORATORY

Head: Prof. Dr. Ren Kimura

Antibacterial Action of Nitro-furan Derivatives

Since 1944 attempts to synthetize nitro-furan derivatives have been undertaken, quite indpendently from Stillman and Dodd, by Dr. H. Saikachi and his collabolators in the Pharmacy Department of Kyoto University. In one of the whole 130 derivatives, i.e. 2-(5-nitro)-furfural semicarbazone, we found in 1946 a predominant antibacterial action against grampositive as well as gramnegative bacteria. The works of Stillman and Dodd were wholly unknown to us at that time.

Since then the antibacterial power in vitro, diffusibility and toxicity of about 60 among 130 synthetized nitro-furan derivatives have been examined in our Institute.

The following is a brief statement of our results:

I. Antibacterial Action in vitro

Serial dilution of each derivative was made with bouillon (pH 6.5) and the minial concentration of growth inhibition of aerobic bacteria was estimated.

1) 5-nitro-furfural derivatives (See Table 1).

The antibacterial power of 5-nitro-furfural semicarbazone (No. 1, which is called "furacin" in the U.S.) is highest of 12 derivatives. The power of No. 2 and No. 3 is inferior to that of No. 1. But Nos. 2 and 3 are different from No. 1, and easily soluble in the water. 5-nitro-furfural phenylsemicarbazone (No. 4) shows a high titer of 1: 320,000 against tubercle bacilli, though it is not very effective, against other bacteria.

2) 5-nitro-furylacrylyl derivatives (See Table 2).

The antibacterial power of 5-nitro-furylacrylyl amide (No. 13) is two times as much as that of furacin. Its solubility is so small as the latter.

3) 5-nitro-furoyl derivatives (See Table 3).

5-nitro-furoylamide (No. 18) is easily soluble in water, and has almost the same antibacterial power as furacin. It is also interesting that thiazole or pyrimidine including derivatives (No. 21, 25 and 31) have strong affinity with staphylococci.

4) 5-nitro-furylacrolein derivatives (See Table 4).

5-nitro-furylacrolein semicarbazone (No. 35) and 5-nitro-furylacrolein aminoguanidine (No. 36) are the most noteworthy derivatives. The former is scarcely soluble in water, but has two times or more antibacterial power as compared with furacin. The antibacterial power of the latter (No. 36) against grampositive bacteria is as strong as the former (No. 35), and it is soluble in water.

5) 5-nitro-furylsulfonamide (See Table 5).

5-nitro-furylsulfonamide (No. 46) has almost the same antibacterial potency as furacin, and is soluble.

6) 5-nitro-furylsulfide (See Table 6).

5, 5′-dinitro-2, 2′-difurylsulfide (No. 52) has strong antibacterial power (1 : 800,000) against staphylococci.

7) Other derivatives (See Table 7).

There are scarcely found any noteworthy derivatives as compared with furacin.

II. Antibacterial Action against Anaerobic Bacteria (See Table 8)

The antibacterial power against Cl. tetani, Cl. oedematis maligni (V. septique) and Cl. novyi of 8 kinds (No. 1, 2, 13, 14, 18, 19, 52 and 53) was examined with the liver-bouillon dilution of the derivatives.

The derivatives have almost the same antibacterial power as homosulfamin.

III. Diffusibility

Each 5 ml of the following composition was poured in a small reagens glass:

1.5% agar	100 ml
1.0% $NaNO_3$	0.5 ,,
0.1% methylene blue	3.5 ,,
Suspension of staphylococci	0.1 ,,

0.5 ml of diluted derivatives was put in the glass, and reagens glasses were kept 18-20 hours in an ice-box and 24 hours in an incubator. The inhibitory length of bacterial growth was estimated.

The result is shown in the Table 9. Nitro-furan derivatives have generally strong diffusibility like penicillin.

IV. Toxicity

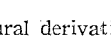
Toxicity of 10 derivatives, expressed in 50% lethal dose per 10 g mouse (Reed and Münch), is given in the Table 10.

5-nitro-furylacrolein semicarbazone (No. 35) is least toxic

V. Fastness Gaining of Bacteria against Nitro-furan Derivatives

The serial cultivation of susceptible strains of Staphylococcus aureus, Escherichia coli communis and Bacillus subtilis was conducted in the 5-nitro-furfural semicarbazone or 5-nitro-furfurylidene aminoguanidine containing media. Each bacterium gains fastness quickly from the 1st to the 10th generation. From the 10th generation on the resistance of the bacteria increases step by step till it reaches the climax in the 25-30th generations. The resistance of Staphylococcus becomes, then, 30 times larger, and that of Escherichia coli and Bacillus subtilis ca. 20 times larger as compared with the original strain. The value of fastness is remarkably inferior to that by penicillin or streptomycin. It is noteworthy that the gained fastness of bacteria against nitro-furan derivatives can not be revived, even if the bacteria are cultivated in drugfree media.

1) Dodd, M. C. & Stillman, W. B.: J. Pharmacol. **82**, 10 (1944).
2) Dodd, M. C.: J. Pharmacol. **86**, 311 (1946).
3) Gramer, D. L. & Dodd, M. C.: J. Bact. **51**, 293 (1946).
4) Eaton Laboratory.: J. A. M. A. **134** (1947).
5) Goi, J.: Kokin Busshitsu Kenkyu, **2**, 40 (1947).
6) Kimura, R., Higashi, N., Goi, J. & Saikachi, H.: J. Penicillin, **2**, 62 (1498).
7) William, C. W. & Dodd, M. C.: J. Bact. **56**, 649 (1948).
8) Takahashi, T., Saikachi, H. *et al.*: Yakugaku Zasshi, **69**, 284 (1949).
9) Takahashi, T., Saikachi, H. *et al.*: *Ibid.* **69**, 286 (1949).
10) Ikegaki, K.: Kokin Busshitsu Kenkyu, **2**, 442 (1949).
11) Ikegaki, K.: *Ibid.* **3**, 15 (1950).
12) Ikegaki, K.: Yakugaku Kenkyu, **22**, 148 (1950).
13) Ikegaki, K.: Kokin Busshitsu Kenkyu, **3**, 179 (1951).
14) Ikegaki, K.: *Ibid.* **3**, 199 (1951).
15) Ikegaki, K.: *Ibid.* **3**, 209 (1951).

Table 1. 5-nitro-furfural derivatives. (unit=10,000, R=NO_2- )

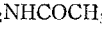
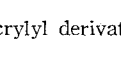
Derivatives \ Bacteria / Hours	Staphylococcus aureus		Streptococcus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 1. R-CH=N-NH-$CONH_2$	20	16	2	1	2	1	20	16	20	10	20	10	<0.5	<0.5
No. 2. R-CH=N-NH·C(NH_2)(=NH)·HCl	10	8	8	4	2	1	5	2.5	10	5	16	8	<0.5	<0.5
No. 3. R-CH=N-NH·C(NH_2)(=NH)·½H_2SO_4	10	8	8	4	2	1	5	2.5	10	5	16	8	<0.5	<0.5
No. 4. R-CH=N-NH-CONH- 	5	2.5					<0.5	<0.5	1	1				
No. 5. R-CH=N-NH-COOH	10	10					2.5	2.5	5	2.5	2.5	2.5	<0.5	<0.5
No. 6. R-CH=N-OH	2.5	1	1	<0.5	1	<0.5	0.5	<0.5	0.5	<0.5	0.5	<0.5	0.5	<0.5
No. 7. R-CH=N-  COOH	2.5	1	1	<0.5	1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
No. 8. R-CH=N-  SO_2NH_2		1						<1		<5		<5		<0.5
No. 9. R-CH=N-  $SO_2NHCOCH_3$		<1		<1						<1				
No. 10. R-CH=N-NH·C(=S)-NH_2		5		<0.5		<0.5		1		1		1		<1
No. 11. R-CH=N-  SC_2H_5	2.5	2.5					<0.5	<0.5	1	1	1	0.5	<0.5	<0.5
No. 12. (R-CH=N-NH-CO)$_2$	2.5	1					<0.5	<0.5	1	1	<0.5	<0.5	<0.5	<0.5

Table 2. 5-nitro-furylacrylyl derivatives. (unit=10,000, R=NO_2- )

Derivatives \ Bacteria / Hours	Staphylococcus aureus		Streptococcus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 13. R-CH=CH-$CONH_2$	40	20	4	2	2	1	40	20	40	20	20	10	1	<0.5
No. 14. R-CH=CH-COOH	20	10	2	1	2	1	10	5	10	5	10	5	<0.5	<0.5
No. 15. R-CH=CH-CONH- 	16	10					2.5	1	10	5	8	5	<0.5	<0.5
No. 16. R-CH=CH-CONH-  Cl	10	8		<0.5		<0.5		<1		<1		<1		
No. 17. R-CH=CH-CONH-  -O-CH_3	10	8					1	0.5	2.5	1				

Table 3. 5-nitro-furoyl derivatives. (unit=10,000, R=NO_2- )

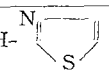
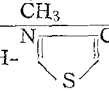
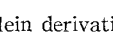
Derivatives \ Bacteria / Hours	Staphylococcus aureus		Streptococcus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 18. R-$CONH_2$	5	2.5	2	1	<1	<1	20	10	20	10	20	10	<0.5	<0.5
No. 19. R-CONH- 	20	10	0.5	<0.5	1	<0.5	5	5	20	10	10	5	<1	<1
No. 20. R-CONH-  COOH	<1	<1	1	0.5	0.5	0.5	1	<1	1	<1				
No. 21. R-CONH- 	10	5					2.5	1	<1	<1	2.5	1		
No. 22. R-CONH-$COCH_3$	<1	<1					2.5	1	5	2.5	2.5	1		
No. 23. R-CONH-  SO_2NH_2	2.5	2.5	<1	<1	<1	<1	<1	<1	2.5	1	2.5	1		
No. 24. R-CONH-  $SONH_2$-$COCH_3$	<1	<1					<1	<1	<1	<1	<1	<1	<1	<1
No. 25. R-CONH-  -S-CH_3	10	5					5	2.5	5	2.5.	2.5	1		
No. 26. R-CONH-  $COOC_2H_5$	5	2.5					<1	<1	<1	<1	<1	<1	<1	<1
No. 27. R-CONH-CH_2COOH	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
No. 28. R-CONH-  -O-C_2H_5	5	2.5					<1	<1	1	<1	2.5	1	<1	<1
No. 29. R-CONH-  -S-C_2H_5	5	2.5					<1	<1	<1	<1			<1	<1
No. 30. R-CONH-CH(CH_3)-COOH	<1	<1					<1	<1	<1	<1				
No. 31. R-CONH- 	20	10					2.5	1	2.5	1				
No. 32. R-CONH-NH-CONH- 	5	2.5					1	<1	1	<1				
No. 33. R-CONH-  -S-  NO_2	No action													

Table 4. 5-nitro-furylacrolein derivatives. (unit=10,000, R=NO_2- )

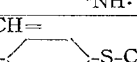
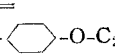
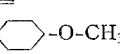
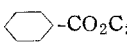
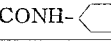
Derivatives \ Bacteria / Hours	Staphylococcus aureus		Streptococcus haemolyticus		Dipococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 34. R-CH=CH-CHO	20	10	<1	<1	<1	<1	10	5	10	5	10	5	<1	<1
No. 35. R-CH=CH-CH=N-NH-$CONH_2$	40	20	10	8	4	2	20	20	20	16	32	16	<1	<1
No. 36. R-CH=CH-CH=N-NH·C(NH_2)(HCl)NH·	32	20	16	8	4	2	2.5	2.5	5	5	10	5	<1	<1
No. 37. R-CH=CH-CH=N-  -$S-C_2H_5$	2.5	1					2.5	1	5	2.5	2.5	1	<1	<1
No. 38. R-CH=CH-CH=N-NH-CONH-  -$O-C_2H_2$	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
No. 39. R-CH=CH-CH=N  -Cl	<1	<1			<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
No. 40. R-CH=CH-CH=N-C_6H_4Cl(m)	1	<1			<1	<1	<1	<1	<1	<1	<1	<1		
No. 41. R-CH=CH-CH=N-  -$O-CH_3$	<1	<1					<1	<1	<1	<1	<1	<1	<1	<1
No. 42. R-CH=CH-CH=N-  -$CO_2C_2H_5$	2.5	1					1	<1	1	<1	1	<1	<1	<1
No. 43. R-CH=CH-CH=N-  OH	10	5	<1	<1	<1	<1	2.5	1	2.5	1	2.5	1		
No. 44. R-CH=CH-CH=N-NH-CONH- 	2.5	1					<1	<1	<1	<1				
No. 45. (R-CH=CH-CH=N)$_2$	<1	<1					<1	<1	<1	<1				

Table 5. 5-nitro-furylsulfonamide derivatives. (unit=10,000, R=NO_2- )

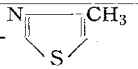
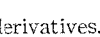
Bacteria / Derivatives / Hours	Staphylococcus aureus		Streptococcus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 46. R-SO_2NH_2	20	10	<1	<1	<1	<1	10	10	20	10	10	5	<1	<1
No. 47. R-SO_2NH- 	2.5	1			<1	<1	1	<1	1	<1	1	<1	<1	<1
No. 48. R-SO_2NH- 	5	2.5			<1	<1	1	<1	1	<1	<1	1	<1	<1
No. 49. R-SO_2NH- 	1	<1			1	<1	2.5	1	2.5	1	<1	<1		
No. 50. R-SO_3K	<1	<1			<1	<1	<1	<1	<1	<1	2.5	1	<1	<1

Table 6. 5-nitro-furylsulfide derivatives. (unit=10,000, R=NO_2- )

Bacteria / Derivatives / Hours	Staphylococcus aureus		Streptococcus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 51. R-S-  -NO_2	10	5	2	1			5	2.5	5	2.5	5	2.5	<1	<1
No. 52. R-S- 	80	60	<1	<1			5	2.5	5	2.5	10	5		

Table 7. Other derivatives. (unit=10,000, R=NO_2- )

Bacteria / Derivatives \ Hours	Staphylococcus aureus		Streptococcus haemolyticus		Diplococcus pneumoniae Type I		Escherichia coli communis		Eberthella typhosa		Shigella dysenteriae		Pseudomonas aeruginosa	
	24	96	24	96	24	96	24	96	24	96	24	96	24	96
No. 53. R-C=C-$COOC_2H_5$ / $COCH_3$	5	2.5			<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
No. 54. R-C=C($COOC_2H_5$)($COOC_2H_5$)	1	<1			<1	<1	<1	<1	<1	<1	<1	<1		
No. 55 R-C≡N	5	2.5	<1	<1	2	1	10	5	10	5	10	5	<1	<1
No. 56. N-CH=NOH	10	5	4	2	2	1	10	5	10	5	10	5	<1	<1

Table 8. Antibacterial action of nitro-furan derivatives against anaerobic bacteria

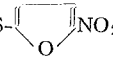
Bacteria / Derivatives \ Hours	Cl. tetani		V. septique		Cl. novyi	
	24	96	24	96	24	96
No. 1. R-CH=N-NH-$CONH_2$	16000	8000	16000	8000	8000	8000
No. 2. R-CH=N-NH·C(NH_2)(=NH·)HCl	16000	16000	16000	8000	8000	4000
No. 13. R-CH=CH-$CONH_2$	16000	8000	16000	16000	8000	<4000
No. 14. R-CH=CH-COOH	8000	8000	16000	8000	8000	4000
No. 18. R-$CONH_2$	8000	4000	6000	6000	4000	<4000
No. 19. R-CONH- 	6000	4000	8000	4000	<4000	<4000
No. 52. R-S- 	8000	6000	6000		<6000	
No. 53. R-CH=C-$COOC_2H_5$ / $COCH_3$	4000	4000	4000	<4000	<4000	<4000

Table 9. Diffusibility of nitro-furan derivatives (mm)

Derivatives \ Concentration	1 : 10,000	1 : 20,000	1 : 40,000	1 : 80,000	1 : 160,000
No. 1. R-CH=N-NH-$CONH_2$	17.0	14.5	12.0	9.0	5.5
No. 2. R-CH=N-NH·C(=NH·)(NH_2)·HCl	17.0	14.5	12.5	9.5	5.5
No. 13. R-CH=CH-$CONH_2$	18.0	15.5	13.0	7.5	4.0
No. 14. R-CH=CH-COOH	14.5	9.0	6.5	3.5	0
No. 18. R-$CONH_2$	9.5	6.0	2.5	0.5	0
No. 30. R-CONH-	14.0	11.0	8.0	5.0	1.0
No. 35. R-CH=CH-CH=N-NH-$CONH_2$		17.5	15.0	11.0	6.5
No. 36. R-CH=CH-CH=N-NH·C(=NH·)(NH_2)·HCl	18.5	16.5	14.5	11.5	7.0
No. 46. R-SO_2NH_2		14.5		9.8	
No. 52. R-S-	23.0	21.5	19.5	17.0	15.0
No. 56. R-CH=NOH	11.0	7.5	3.5	1.5	0

Table 10. 50% lethal dose per 10 gr mouse (mg)

	subcutan.	per os
No. 1. R-CH=N-NH-$CONH_2$	6.25	2.08
No. 2. R-CH=N-NH·C(=NH·)(NH_2)·HCl	1.2	1.43
No. 13. R-CH=CH-$CONH_2$	3.13	1.88
No. 14. R-CH=CH-COOH	4.35	2.12
No. 18. R-$CONH_2$	1.46	1.88
No. 35. R-CH=CH-CH=N-NH-$CONH_2$	11.11	9.26
No. 36. R-CH=CH-CH=N-NH·C(=NH·)(NH_2)·HCl	1.0	1.5
No. 46. R-SO_2NH_2	2.18	2.5
No. 53. R-CH=C-$COOC_2H_5$ ($COCH_3$)	1.25	
No. 56. R-CH=NOH	0.31	